INTRODUCTION

The reaction of amino acids, proteins, peptides, imino acids and amines with triketohydridine hydrate (ninhydrin) is of special importance for their detection and estimation. The ninhydrin reaction is widely used to disclose the location of amino acids on paper and thin layer chromatograms by spraying with a solution of ninhydrin followed by heating. The reaction is also used in automatic amino acid analyzers developed at Rockefeller Institute in 1950 and since then have become commercially available. The reaction with amino acids has been described in almost all text books\(^1\)\(^-\)\(^5\) of organic chemistry and biochemistry. Two aspects of the reaction, related with its analytical uses and with its kinetics and mechanism, have been studied and discussed in literature and are described below:

Studies of ninhydrin reaction related with its analytical uses:

(1) Colorimetric estimations of amino acids and related compounds

The colour reaction between ninhydrin and amino acids was discovered by Ruhemann in 1910. Ruhemann\(^6\)\(^-\)\(^7\) found that amino acids reacted with ninhydrin to give a purple colour in aqueous medium, known as the "Ruhemann's purple" and was used for the colorimetric estimation of amino groups. Harding and
MacLean\textsuperscript{8-9} studied the reaction under different conditions and it was found that stable colour could be obtained in excess of ninhydrin in presence of pyridine. These authors showed that the estimation of $\alpha$-nitrogen of amino acid by the method was quite satisfactory within the range of 0.005 mgm to 0.05 mgm per cubic centimeter. One ml of a 0.1 percent solution of the amino acid was added to 0.5 ml of a 1 percent solution of triketohydridinehydrate, and 0.2 ml of freshly distilled pyridine was added, and the mixture was heated in a boiling water bath for varying intervals of time. The estimation of relative amounts of coloring matter was carried out by comparison in a Duboscq colorimeter. It was found that the reaction between amino acid and triketohydridinehydrate in presence of pyridine took place rapidly and the colour attained a maximum value in about twenty minutes. A determination was made of the relative amounts of colouring matter produced by alanine, glycine, aspartic acid and glutamic acid. These workers also studied the reaction of ninhydrin with the amines and amides. It was found that amides, guanidine and its derivatives gave negative test with ninhydrin.

Moore and Stein\textsuperscript{10} studied the reaction of ninhydrin with amino acid for the use in chromatography. The reaction of ninhydrin with $-\text{NH}_2$ groups to give diketohydrindylidene-diketohydrindamine gained new importance because of its
utilization as the basis for a photometric determination of amino acids and related compounds in effluent samples from starch chromatograms. The colour yield had been rendered fully reproducible by the incorporation of hydantoin or stanous chloride in the reagent solution to eliminate oxidative side reactions. Although the colour yield from a given amino acid was constant, the different amino acids do not give the same percentage yield of the blue product. This fact does not prevent accurate use of the method in chromatographic work in those cases where the individual amino acids are separated from one another by the fractionation process. The reaction was carried out at pH 5 and 100°C and the absorption maximum of the blue product was found at 570 nm. For individual amino acids the accuracy was 2 percent for samples in the range of 2.5 of α-NH₂ nitrogen.

The mechanics of the procedure was developed to permit the analysis of a large number of samples on a routine basis.

Troll and Cannan modified the procedure for the photometric determination of amino acids with ninhydrin using different organic solvents and found that ten of the naturally occurring amino acids gave theoretical yields of colour at room temperature. At 100°C all amino acids, except tryptophan and lysine reacted quantitatively. They recommended the following method for the analysis of the primary amino acids.
Reagents:

(1) Ninhydrin solution: 500 mg of ninhydrin was dissolving 10 ml of absolute alcohol.

(2) 80 percent phenol solution: 80 gm. of reagent grade phenol was dissolved in 20 ml of absolute alcohol with gentle heating. The solution was shaken with 1 gram of permutit for about 20 minutes to remove traces of ammonia and then decanted.

(3) KCN-pyridine reagent: 2 ml of 0.01M solution of KCN was diluted to 100 ml with ammonia free pyridine, prepared by shaking 100 ml of pyridine with 1 gm. of permutit for about 20 minutes.

(4) 60 percent alcohol (by volume).

Procedure:

0.4 to 0.5 ml of aqueous amino acid solution containing 0.05 to 0.5 μm of the amino acid was heated with 1 ml of KCN-pyridine reagent and 1 ml of 80 percent phenol reagent in the boiling water bath. After the attainment of the temperature of the bath, 0.2 ml of the ninhydrin solution was added. The solution was cooled and made up to 10 ml with 60 percent alcohol and the optical density at 570 nm was measured. These authors also described a method for the
photometric estimation of hydroxy-proline based on the extraction of red derivative from the reaction system with benzene which is a precursor of the yellow compound which is the final product of the reaction with ninhydrin. Troll and Lindsley described a photometric method for the estimation of proline and its application to protein hydrolysates, urine and plasma. Piez, Irreverre and Wolff described two colorimetric procedures for the quantitative estimation of cyclic imino acids in the effluent fractions from an ion exchange column. Both employed ninhydrin in glacial acetic acid. When the reaction was carried out at room temperature, the method was suitable for hydroxy proline, allohydroxyproline and proline. Heating at 100°C permitted the determination of 5-hydroxypipecolic acid, proline, baikain and pipecolic acid. The separation of these compounds by ion exchange and paper chromatography was also described.

Moore and Stein extended their studies on the chromatography of amino acids on columns of ion exchange resins for the determination of amino acids and related compounds with ninhydrin. They developed a modified reagent composed of 2 percent ninhydrin and 0.3 percent hydridantin and 3:1 methylcellosolve-4N sodium acetate buffer (pH 5.5). The method was used for the analysis of effluent fractions
obtained in ion-exchange chromatography. Yanari\textsuperscript{15} studied the reaction of ninhydrin with various dipeptides at 100°C and pH 5. Several fold differences in rate were observed in the reaction of ninhydrin with the diestrioiisomeric pair but equal rates were approached when the concentration of methylcellosolve or dioxane was increased. High methylcellosolve concentrations favoured more rapid and complete reaction of dipeptides.

Yemme and Cocking\textsuperscript{16} adapted the method of Troll and Cannan for routine use. Their procedure involved the preparation of cyanide-ninhydrin solutions, which are also unstable. Rosen\textsuperscript{17} modified the method by avoiding the prior preparation of an unstable ninhydrin reagent. 3 percent ninhydrin solution was prepared in methyl cellosolve separately. Sodium cyanide (0.01M) acetate buffer (2700 gm NaOAc.3H₂O + 2 litre H₂O + 500 ml glacial HOAc make up to 7.5 litre with H₂O) was adapted from that of Stein and Moore and its pH should be 5.3 - 5.4. Acetate cyanide (0.0002M NaCl in acetate buffer) was prepared by diluting cyanide solution fifty times with acetate buffer. The estimation of the amino acids was done by mixing the reagents in usual way.

Rosen, Berard and Levenson\textsuperscript{18} used the method for the automatic analysis of amino acids by ninhydrin reagent. The basic modifications are alterations in the ninhydrin reagent
and addition of cyanide in buffer system which rendered the reagent stable indefinitely in light and air and eliminated the risk of clogging.

Kirschenbaum\textsuperscript{19} used dimethylsulfoxide in a ninhydrin reagent along with methyl cellosolve (60% methylcellosolve - 40% dimethyl sulfoxide). This combination of the solvent minimized the precipitate formation in the capillary lines of amino acid analyzer. Moore\textsuperscript{20} completely replaced methylcellosolve and used dimethylsuloxide as the solvent. It was found that dimethyl sulfoxide is a better solvent for the reduced form of ninhydrin (hydrindantin) than methylcellosolve. Better performance and improved stability was observed with dimethyl sulfoxide. The result was a ninhydrin hydrindantin solvent in 75% DM30-25% 4M lithium acetate buffer at pH 5.2. The reagent was prepared for the amino acid analyzers operated under the general conditions described by Spackman, Stein and Moore\textsuperscript{21}. 3 litre of dimethylsulfoxide and 1 litre of the 4M lithium acetate buffer were added to the 4 litre filling bottle, after nitrogen had been bubbled through dimethylsulfoxide buffer mixture for 15 minutes, 80 gm of ninhydrin was added and than 2.5 gm of hydrindantin was dissolved. The reagent gave yields of colour in the ninhydrin reaction with amino acids quite identical with the reagent prepared in the methylcellosolve.
Yamamoto and Young\textsuperscript{22} prepared hydridantin-ninhydrin reagents with Ni-Pt electrodes electrochemically and also by SnCl\textsubscript{2} reduction. They compared the colour yields of more than 30 ninhydrin positive compounds including nor-leucine as an internal standard and concluded that the two methods of reducing ninhydrin did not yield equivalent colour constants and therefore to avoid potentially gross errors, it was necessary to determine colour constants on every compound of interest for each different method of preparing hydridantin-ninhydrin reagent.

James\textsuperscript{23} studied the colour development of amino acids with ninhydrin reagent prepared by reduction with titanous chloride. They compared their results with stannous chloride and found that titanus chloride is superior reducing agent for ninhydrin reduction.

Pochinok\textsuperscript{24} developed new reagent for the estimation of amino acids. The reagent consisted of ninhydrin dissolved in ethylene glycol with an addition of butyl alcohol and propyl alcohol. The reagent was more stable and had no unpleasant odour. It permitted determination of 0.2 - 2.0 \( \mu \)g of amino nitrogen per milli litre of solution. Bell and Mason\textsuperscript{25} developed rapid chromatographic method for the estimation of lysine using acid ninhydrin reagent and measuring the optical density of the yellow lysine-ninhydrin complex
at 440 nm. The method was further extended by Devi to the determination of lysine and ornithine using acid ninhydrin. The optical density was measured at 440 nm and 515 nm for lysine and ornithine respectively. Sakari and Koji studied the effect of L-ascorbic acid on the colorimetric estimation of amino acids with ninhydrin. Friedman and Williams determined keratin proteins with ninhydrin and found that dimethylsulfoxide-water (40-60% by volume) was the best solvent.

The ninhydrin reagent was developed and modified for spectrophotometric determination of amino acids by liquid chromatography by a number of workers. The studies were mainly related with the effect of different reducing agents and pH. The ninhydrin reagent was also modified to be used in automatic amino acid analyzers on the same lines by using different organic solvents.

Seiji, Kazunori and Yoshijiro studied the effect of metal complexing agents (without reducing agents) and ninhydrin on the chromatographic determination of amino acids and related compounds. The reaction was carried out at 135-200°C. It was observed that at high temperature ninhydrin reacted with amino acids and related compounds even without reducing agents. Singh, Khanna and Singh observed that Mn²⁺, Fe²⁺ and Mo²⁺ increased the intensity
of colour product formed by the interaction of ninhydrin with amino acids and Cd$^{2+}$, Zn$^{2+}$ and Al$^{3+}$ depressed the colour formation. Ganapathy, Ramchandramurty and Radhakrishnan$^{49}$ developed a Cu$^{2+}$ ninhydrin reagent to distinguish qualitatively a small α-peptides and α-amino acid amide from free amino acids by paper chromatography. It was suggested that Cu$^{2+}$ first formed complex with peptides and than reacted with ninhydrin to give a yellow chromophore.

**Estimation of amino acids by the measurements of evolved CO$_2$**

The utilization of ninhydrin for the quantitative determination of amino acids has been adapted in several ways. Ruhemann$^{50}$ and Grassmann and Von Arnin$^{51}$ showed that the colour forming reaction of ninhydrin with amino acids was accompanied with the evolution of Carbon dioxide. Both Van Slyke and Dillon$^{52}$ and Mason$^{53}$ devised methods for the determination of amino acids based on the measurements of the carbon dioxide evolved. These investigators adapted manometric procedures as developed by Van Slyke$^{54-55}$ and others for the determination liberated carbon dioxide. Christensen$^{56}$ developed a very simple apparatus for the measurements of the evolved CO$_2$ which had been successfully applied in several ways and was also used for the determination of amino acids with ninhydrin. The time required for a complete determination was only twenty minutes.
Van Slyke, MacFadyen and Hamilton\textsuperscript{57} described a titration method determining free amino acids by titration of the carbondioxide evolved from carboxyl groups during reaction with ninhydrin. The evolved CO\textsubscript{2} was transferred in standard barium hydroxide solution and titrated. The results are comparable with those obtained from manometric techniques. Van Slyke, Dillon, MacFadyen and Hamilton\textsuperscript{58} described a method for the gasometric determination of carboxyl groups in free amino acids. The method was based on the fact that \( \alpha \)-amino acids, when boiled in water with an excess of ninhydrin at pH 1-5, evolved CO\textsubscript{2} from their carboxyl groups quantitatively in few minutes. The reaction was found to be specific for free amino acids because it required the presence, in the free unconjugated state, of both the carboxyl and the neighbouring-NH\textsubscript{2} or NH-CH\textsubscript{2} groups. Other reagents were also used for the evolution of CO\textsubscript{2} from \( \alpha \)-amino acids, but ninhydrin was found to give the most precise and specific results.

Maso\textsuperscript{63} et al. studied the reaction of amino acids with glyoxal and ninhydrin to give carbondioxide in acetate buffer (0.5M, pH 5 at 50\(^{\circ}\)C). The generation of carbondioxide was found to be linear with time. Steric and polar effects had an important role in the decarboxylation reaction which was dependent on the anion concentration of amino acid.
Kinetic and mechanistic studies of ninhydrin reaction

Ruhemann's view on the interaction of amino acids and triketohydindine hydrate involved the oxidation of the amino acids to carbon dioxide and an aldehyde possessing a carbon atom less than the amino acid with the simultaneous reduction of the triketone to hydrindantin and the condensation of the hydrindantin with the ammonia liberated by the oxidation of amino acid, forming the blue coloured ammonium salt of diketohydindilidine-diketohydindamine. His mechanism for the reaction is given in Scheme 1. In modern terms the purple colour would be associated with the anion rather than the ammonium salt. The first stage of the reaction (1) and the last (3), in which hydrindantin reacts with two molecules of ammonia in a most unlikely manner, are not discussed. The theory can not account for the more rapid chromogenic reaction of $\alpha$-amino acids with ninhydrin and with hydrindantin, as compared with amines and ammonium salts. His suggestion as to the origin of Ruhemann's purple is subsequently correct.

Harding and Warneford studied the ninhydrin reaction with amino acids and ammonium salts and utilized the theory of Dakin and Dudley, who had postulated that amino acids undergo either a dissociation or a decomposition into ammonia and the corresponding glyoxal. Methyl glyoxal
and ammonia is formed in case of alanine. Methyl glyoxal is a powerful reducing agent and could reduce triketohydrindene hydrate to 1,3-diketohydrindol which on condensation with the ammonia from the amino acid would give 1,3-diketohydrindamine. This amine readily condense with aldehydes and ketones, is readily oxidised to deep blue colouring matter, and consequently would be expected to condense with a molecule of triketohydridinehydrate to give diketohydrindylidine-diketohydrindamine, the ammonium salt of which is the required blue colouration. The mechanism is given in Scheme 2. The advantage of this interpretation is that it explains why ammonium salts are able to produce a purple colour. However, since ammonia is postulated as an intermediate, the theory does not explain why amino acids react faster with hydrindantin than does ammonia. Negligible amount of ammonia is evolved from amino acids in the absence of ninhydrin, and the source of carbon dioxide, which is evolved, can not be the α-keto acid. It has been shown that the evolution of carbon dioxide from α-ketoacids is much slower than from amino acids in the presence of ninhydrin.

On the basis of experimental work Retinger rejected the previous theories of ninhydrin reaction and proposed another one as given in Scheme 3. According to him the triketohydrindene hydrate hydrolyses during boiling giving O-carboxyl glyoxal which reduces part of the triketohydrindene
to dioxindane, which in turn combines with another molecule of triketohydrindene to give hydrindantin. The amino acid or amine derived from enzyme action gives first a monobasic salt which is colourless, further boiling produces a dibasic neutralization, and the molecule splits into equal parts with trivalent carbon - a free valency is cause of the absorption in the visible spectrum. Exposure to air in water solution decomposed the split molecules further, giving O-carboxylmandelic acid, ammonia, carbon dioxide, water, aldehyde and dioxindene through reduction. Retinger's theory has a number of limitations, the most important being that salts of hydrindantin show no absorption in the visible range of the spectrum$^{72,73}$ and that the visible and ultraviolet absorption spectra of Ruheman's purple and hydrindantin differ greatly in alkaline solution.

Worker and Antener$^{67}$ have also studied the reaction of ninhydrin with amino acids and have proposed the mechanism given in Scheme 4. They subjected less likely ethylenimine structures for Ruhemann's purple without any experimental evidence.

Schonberg$^{68}$ et al., studied the Strecker's degradation of $\alpha$-amino acid and amine with carboxyl compounds to give the corresponding aldehydes and ketones with one less carbon atom. They suggested that the following two conditions must be satisfied.
1. Two hydrogen atoms attached to the nitrogen atom must be unsubstituted.

2. The carbonyl compound must contain the grouping
   \(-\text{CO-} \overset{\text{CH=CH}_n}{\text{-CO-}}\) where \(n = 0\) or an integer, and that at least one CO group must be aldehydic or ketonic.

The mechanism proposed is given in Scheme 5. In the Scheme 5 structures I & II are isomers according to classical view, but they may be considered as extreme structures of a molecule having the nature of a resonance hybrid; the theory of hydrogen bonding should be applied. It is possible that the ease with which the degradation proceeds in a number of cases is due to the fact that the intermediate Schiff's base, being hybrids, are ketonic as well as enolic.

Badder\(^6\) also studied the Strecker degradation of amino acids with carbonyl compounds and had suggested an electronically interpreted mechanism (Scheme 6). The mechanism was severely criticized on the grounds that he had provided insufficient experimental evidence.

Moubasher and Ibrahim\(^7\) have studied the reaction between ninhydrin and \(\alpha\)-amino acids, and realized that the ninhydrin reaction was a special case of the much more general strecker reaction. They proposed the formation of hydrindantin and bis-1,3 diketoindanyl, the latter compound arised from the former. Hydrindantin possibly formed by the condensation of ninhydrin with 2-hydroxy 1,3 diketoindane. The mechanism proposed is given in Scheme 7. In support of
(Scheme 7) mechanism, only two new experimental observations are offered. 2,2-Bis(1-Hydroxy-3-Ketoindene) a dark brown material, was obtained from the reaction of ninhydrin or hydrindantin with alanine. As far as the formation of hydrindantin their scheme follows the normal route, but beyond this point the mechanism is conjectural. No spectral data are offered, and in fact there is little resemblance between the absorption curve of 2,2-Bis(1-Hydroxy-3-Ketoindene) and those of aqueous solutions of ninhydrin and amino acids after reaction.

MacFadyen\textsuperscript{71-72} have shown that the formation of purple colour in the amino acid-ninhydrin reaction is independent of the nature of the cation and is to be attributed to the anion of diketohydrindamine-diketohydrindylidine (Ruhemann's purple) as given below:

![Diketohydrindamine-Diketohydrindylidine](image)

It is not an ammonium salt as suggested by Ruhemann\textsuperscript{6,7} and Harding and MacLean\textsuperscript{8,9}.

MacFadyen and Fowler\textsuperscript{73} in their studies of the reaction of ninhydrin and hydrindantin with $\alpha$-amino acids summarized the observations of some workers on the basis of their studies. They arrived at the following conclusions.
1) The red and blue colours formed by dissolving hydrindantin in alkaline solution have been studied spectrophotometrically under controlled conditions of oxygen content, pH and temperature. The monovalent and bivalent anions of indanone-enediol are represented by red and blue colours respectively.

2) At pH 5.3, the sodium salt of diketohydrindamine-diketohydrindylidine formed in the reaction of α-amino acids with hydrindantin but ammonia is not formed as the intermediate. Ruhemann's purple is formed in the reaction by using mole for mole of indanone-enediol in the reaction.

3) The mechanism of the reaction of amino acids and other amines with ninhydrin proposed earlier were not supported by the studies except the concept about the ammonium salts put forward by Harding and Warenford and Harding and MacLean. Filippovich proposed the mechanism (Scheme 8) for the reaction between α-amino acids and ninhydrin. Step I is simply the widely known reaction of the amino group of the amino acid with carbonyl group to form the Schiff base. The high mobility of the carbonyl oxygen of the middle oxo-group of ninhydrin permits such a reaction. Step II is similar to one of the steps of reamination and the decarboxylation of step III is fully natural. The reaction of self forming "ninhydrinolysis" in step IV is not only probable, but necessary if the stability of the colouration in the presence
of ninhydrin in the reaction medium is taken into account. Step V had already been proposed. The mechanism has not given the formation of NH$_3$ and hydrindantin.

McCaldin$^{75}$ has reviewed the chemistry of ninhydrin in detail describing its synthesis, structure and properties. He also gave a detail account for the reaction of ninhydrin with alkalies and amino acids. The different theories of the formation of Ruhemann's purple were described and a mechanism was proposed (Scheme 9). The mechanism does not involve hydrindantin in the colour formation pathways, but involves the compound in a side reaction and does not explain the role of reducing agents$^{10}$ in the colour formation. McCaldin also described the reaction of ninhydrin with imino acids, amines (primary, secondary, tertiary), pyrroles and ammonium salts. Ninety eight references were quoted in the review.

Lagercrantz and Yhland$^{76}$ detected the free radicals in some reactions of ninhydrin by ESR techniques. It was concluded that there is no correlation between the radical concentration and the purple colour developed in the reaction between ninhydrin and amino acids. No radicals could be detected in an aqueous solution below pH 7.

Friedman and Sigel$^{77}$ studied the kinetics of the ninhydrin reaction with amino acids and measured the rate of colour development at 30 and 100°C. It was shown that the
reaction rate is a function of basicity and steric environments of the amino groups. A linear free energy equation was also derived on the basis of observed reactivity of α-amino acids at 30°C, that permits calculation of separate polar and steric parameters associated with the amino acids which influence the rates. They proposed that rate determining step in the ninhydrin reaction appears to involve a nucleophilic type displacement of hydroxy group of hinhydrinhydrate by a nonprotonated amino group.

Lamothe and MacCormick studied the influence of acidity on colour production in the reaction of ninhydrin with amino acids. A thorough study, using the Hammett acidity function was presented on the problem of pH measurements in the partially aqueous system in which colour is developed. The effect of change of pH of the aqueous buffer on colour production for twenty common amino acids was studied. The relationship between optimum pH and pK for amino acids could be found by substitution of the dissociation constant expression into the second order rate equation. These workers further studied the role of hydrindantin in the colorimetric determination of amino acids using ninhydrin at 40°C and the proposed mechanism is given in Scheme 10.

Johnson and McCaldin proposed the structure for the yellow and purple condensation products from ninhydrin with cyclic α-imino carboxylic acid and discussed the influence
of ring size on the course of reaction. It was also reported that purple condensation product is also obtained from ninhydrin and cyclic bases and the mechanism of these condensations was discussed.

Moubasher and Othman discussed the interaction of cyclic triketone and amines which react to give aldehyde, ammonia and the corresponding reduction product of triketone. The suggested mechanism is given in Scheme 11. These authors discussed the structure of alloxantin and concluded that the evidence available favours the pinacole rather than hemiacetal formation. Some reactions of alloxan and hydrindantin with amines and amino acids were described.

Ahmad and Ahmad described the reaction of ninhydrin with n-substituted anilines and n-substituted guanidine and proposed the structure for the product and also gave the probable mechanism for the reaction. Friedman and Williams studied the reaction of ninhydrin with amines which has both bioanalytical and bioorganic significance and explained the stoichiometry on the basis of slow formation, side reactions, hydrolytic, oxidative and photolytic instability and interfering colour.

Yu and Davis studied deuterium isotope effects in the ninhydrin reaction of primary amines and found that the rate of development of Ruhemann's purple in the ninhydrin reaction of αα-d₂-p-tyramine and αα-d₂-β-phenylethylamine is
significantly reduced. It appears to be a primary isotope effect and indicates that the cleavage of C-H bond at the $\alpha$-position is involved in the rate determining step of the colour reaction. The proposed mechanism for primary amines and $\alpha$-amino acids is given in Scheme 12.

Malaviya and Katiyar$^{86}$ studied the mechanism of acid hydrolysis of Ruhemann's purple produced by the reaction of amines with ninhydrin, and investigated the effect of micelles on its rate. The effect of pH, ionic strength and organic solvents was investigated and a mechanism was proposed.

Ronald Grigg$^{87}$ et al. carried out trapping experiments with dipolarophiles which provide evidence for the formation of 1,3-dipoles as intermediate in the ninhydrin reaction. It was shown that protonated Ruhemann's purple is a stable N-protonated 1,3 dipole by trapping experiments and by X-ray crystal structure determination.
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\text{Ruhemann, S., J. Chem. Soc., 99, 792, 1486 (1911)}
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Scheme 2

SCHEME - 3

Scheme 6
Moubasher, R, and Othman, A.M. J.A.M. Chem. Soc. 72, 2666 (1950)

**Scheme - 12**
STATEMENT OF THE PROBLEM

On the basis of the survey of the literature on the interaction of amino acids with ninhydrin. It was concluded that the proposed mechanisms are quite complicated and are not supported by systematic kinetic studies. It was, therefore, thought worthwhile to carry out systematic studies on the kinetics and mechanism of the reaction. The evolution of carbon dioxide, the formation of the Ruhemann's purple, hydrindantin and ammonia, have been reported under different conditions. It was planned that kinetic studies should be carried out according to the following scheme:

(1) Studies on the kinetics and mechanism of decarboxylation of amino acids with ninhydrin.

The decarboxylation reaction was studied under the following conditions.

(a) Effect of ninhydrin concentration on the rate of carbon dioxide evolution.

(b) Effect of temperature ionic strength and pH.

(c) The effect of polar and steric factors using different amino acids.

(2) Kinetic studies related with the development of colour, formation of ammonia and hydrindantin in the interaction of ninhydrin with amino acids.

The colour formation and evolution of ammonia were carried out under the following conditions.
(a) Effect of ninhydrin concentration.
(b) Effect of temperature.
(c) Effect of ionic strength and pH.
(d) Effect of solvent.

A qualitative study of the formation of hydrindantin was also done.